FORM PTO-1390 (REV. 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
0230-0162P	09/856717

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP99/06617	November 26, 1999	November 27, 1998

TITLE OF INVENTION

γδT CELL IMMUNOACTIVITY ENHANCERS CONTAINING EXTRACT OF LENTINUS EDODES MYCELIUM

APPLICANT(S) FOR DO/EO/US

ASANO, Kenji; MATSUDA, Yukiko and TAJIMA, Yutaka

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau. WO 00/32213
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is transmitted herewith.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4)
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☒ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 20. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.-1449 and International Search Report w/ cited references
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information:
    PCT/ISA/210
    PCT/IPEA/409
    PCT Request

JC18 Rec'd PCT/PTO 25 MAY 2001

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/856717	PCT/JP99/06617	0230-0162P

21. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. **$1,000.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO. **$710.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4). **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	15 - 20 =	0	X **$18.00**	$ 0	
Independent Claims	3 - 3 =	0	X **$80.00**	$ 0	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)		Yes	+ **$270.00**	$ 270.00	
			TOTAL OF ABOVE CALCULATIONS =	$ 1,260.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				$ 0	
			SUBTOTAL =	$ 1,260.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)).			+	$ 0	
			TOTAL NATIONAL FEE =	$ 1,260.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property			+	$ 0	
			TOTAL FEES ENCLOSED =	$ 1,260.00	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of $ **1,260.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account. No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-2448.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

Send all correspondence to:
Birch, Stewart, Kolasch & Birch, LLP or Customer No. 2292
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Date: May 25, 2001

By _____ #32,977
Gerald M. Murphy, Jr., #28,977

/rem

PATENT
0230-0162P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	ASANO, Kenji et la.	Conf.:	
Int'l. Appl. No.:	PCT/JP99/06617		
Appl. No.:	NEW	Group:	
Filed:	May 25, 2001	Examiner:	
For:	γδT CELL IMMUNOACTIVITY ENHANCERS CONTAINING EXTRACT OF LENTINUS EDODES MYCELIUM		

PRELIMINARY AMENDMENT

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, DC 20231

May 25, 2001

Sir:

The following Preliminary Amendments and Remarks are respectfully submitted in connection with the above-identified application.

AMENDMENTS

IN THE SPECIFICATION:

Please amend the specification as follows:

Before line 1, insert --This application is the national phase under 35 U.S.C. § 371 of PCT International Application No. PCT/JP99/06617 which has an International filing date of November 26, 1999, which designated the United States of America and was not published in English.

IN THE CLAIMS:

Please amend the claims as follows:

9. (Amended) The γδT cell activity enhancer of claim 1 used for treating tumor.

10. (Amended) The γδT cell activity enhancer of claim 1 used for preventing or treating a bacterial infection or a viral infection.

REMARKS

The specification has been amended to provide a cross-reference to the previously filed International Application. The claims have been amended to delete improper multiple dependencies and place the application into better form for examination.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By #32,968
for Gerald M. Murphy, Jr., #28,977

GMM/rem
0230-0162P

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(Rev. 02/12/01)

VERSION WITH MARKINGS TO SHOW CHANGES MADE

The claims have been amended as follows:

9. (Amended) The γδT cell activity enhancer of[any one of claims 1 to 8] claim 1 used for treating tumor.

10. (Amended) The γδT cell activity enhancer of[any one of claims 1 to 8] claim 1 used for preventing or treating a bacterial infection or a viral infection.

SPECIFICATION

γδT CELL IMMUNOACTIVITY ENHANCERS CONTAINING EXTRACT OF LENTINUS EDODES MYCELIUM

FIELD OF THE INVENTION

The present invention relates to the development and preparation of γδT cell activity enhancers and therefore immunopotentiators containing an extract of Lentinus edodes mycelium.

The present invention also relates to the development and preparation of foods, drinks and feeds containing an extract of Lentinus edodes mycelium and having a γδT cell activity-enhancing effect and therefore an immunopotentiating effect.

The present invention also relates to the development and preparation of antitumor agents, therapeutic agents against bacterial infections and therapeutic agents against viral infections containing an extract of Lentinus edodes mycelium.

PRIOR ART

Characteristics of γδT cells

Peripheral T cells in animal blood are mainly classified into two groups based on the type of their cell surface antigens referred to as T cell receptors (TCR). One type is an αβT cell bearing TCR α and β chains on their cell surfaces, and the other is a γδT cell bearing TCR γ and δ chains. γδT cells are cytotoxic killer cells present at a level of only a few to about 10% in normal peripheral blood

and lymphoid tissue in humans, and the characteristics of which are quite distinct from those of αβT cells.

In humans, γδT cells are present in the intestinal tract, skin and peripheral blood or the like and elicit a local immunity. So far, γδT cells have been reported to have functions such as cytotoxicity against cancer cells, and protective activity against bacterial or virus infections, etc.

Phylaxis activity of γδT cells

Some γδT cells in the spleen and other organs produce cytokines such as IL-4 or IFN-α in response to infection. It has been shown under experimental conditions that when these cells are lacking, resistance to bacterial infections is reduced. For example, there is one report which describes that resistance to Mycobacterium tuberculosis infection decreased in mice treated with a γδ-type TCR antibody to transiently inhibit functions of γδT cells or mice deficient in the TCR γ gene (Ladel C. et al., Eur. J. Immunol., 1995, 25:2877-2881). Another report describes that γδT cells appear during the early stage of infection with Listeria monocytogenes (Hiromatsu K. et al., J. Exp. Med., 1992, 175:49-56). These findings suggest that γδT cells play an important role in protecting against bacterial infections.

It has also been reported that chronic hepatitis B virus infection induces the growth of γδT cells in the liver and spleen (Ozaki S. et al., J. Med. Invest., 1998,

44:215-217); and that vaccinia virus markedly increases during the early stage of infection in γδT cell-deficient mice as compared with normal mice (Welsh RM, et al., Immunol. Rev., 1997, 159:79-93). These findings suggest that γδT cells act not only on bacterial infections but also on viral infections.

Cytotoxicity of γδT cells against cancer cells

γδT cells are a class of T cells that are capable of specifically targeting and killing autologous cancer cells, but show no cytotoxicity to autologous normal lymphocytes (such as αβT cells). In this respect, in cancer therapy which employs activated γδT cells there is very little danger of side effects. In contrast to γδT cells, αβT cells are known to kill autologous leukocytes rather than autologous cancer cells, and thus in cancer therapy which employs activated αβT cells there is a likelihood that serious side effects will occur. In view of this, cancer therapy which employs activated γδT cells is desirable.

Moreover, γδT cells have similar characteristics to NK cells such as their MHC-nonrestricted cytotoxicity against cancer cells. γδT cells are present in peripheral blood of children at about 10% but decrease with age. This suggests that the increase in the occurrence of cancer with age may be related to a decrease in γδT cells. In peripheral blood of chicken, sheep, cow or the like, γδT cells are found at levels as high as 15-50%. The low incidence of tumors in these animals suggests that the

presence of peripheral $\gamma\delta$T cells may contribute greatly to the inhibition of cancer.

Pharmacological effects of Lentinus edodes

Lentinus edodes (Shiitake) is a common edible mushroom found in both Japan and China, and has been cultivated in Japan for around 300 years. The part of the mushroom used as food econsists of the reproductive body, also referred to as the fruiting body of fungi, which produces sporesfor reproduction, while the vegetative body includes hyphae which produce mycelia extending into a growing area such soil or logs.

Lentinus edodes has long been believed to have some effect against a variety of diseases and symptoms, but it is only relatively recently that any pharmacological effect has been elucidated. Various effects of extract of Lentinus edodes mycelium are reported, these include the inhibition of oncogenesis in the large bowel and liver and the growth of transplanted tumor cells and increased survival of animals in carcinogenesis experiments in rats and mice (N. Sugano et al., Cancer Letter, 27:1, 1985; Y. Suzuki et al., Journal of the Japan Society of Coloproctology, 43:178, 1990, etc.); mitogenic activity (T. Tabata et al., Immunopharmacology, 24:57, 1992; Y. Hibino et al., Immunopharmacology, 28:77, 1994, etc.); enhanced antibody production and inhibitory effects against immunological hepatocyte damage caused by ADCC (antibody-dependent cell-mediated cytotoxicity) (Y. Mizoguchi et al., Journal of Hepato-Biliary-Pancreatic Study, 15:127, 1987).

These findings have acted as a catalyst for concentrated research into the pharmacological effects of ingredients of Lentinus edodes in the medical and pharmaceutical field. As a result, researchers have discovered that some of the constituents of Lentinus edodes can be used in the treatment of cancer or other diseases by restoring immune function in humans, and further that such constituents may also inhibit the onset of cancer.

In accomplishing the present invention the inventors have aimed to further elucidate pharmacological effects of an extract of Lentinus edodes mycelium, and also to search for new applications of the extract in the form of drugs, foods, drinks, feeds, etc.

One object of the present invention is to develop and provide a γδT cell activity enhancer and therefore an immunopotentiator such as an antitumor agent, therapeutic agent against bacterial infections and therapeutic agent against viral infections containing an extract of Lentinus edodes mycelium.

Another object of the present invention is to use a γδT cell activity enhancer containing an extract of Lentinus edodes mycelium or an immunopotentiator containing an extract of Lentinus edodes mycelium to treat a tumor in a subject.

DISCLOSURE OF THE INVENTION

As a result of intensive studies aimed at solving the above problems, the present invention has been accomplished

on the basis of the finding that an extract of Lentinus edodes mycelium exhibits an effect of remarkably enhancing the activity of γδT cells.

Accordingly, the present invention has been developed and provided γδT cell activity enhancers and therefore immunopotentiators such as antitumor agents or therapeutic agents against bacterial or viral infections containing the extract of Lentinus edodes mycelium.

The present invention also provides methods developed for treating tumor, bacterial infections and viral infections using the extract of Lentinus edodes mycelium.

γδT cell activity enhancers or immunopotentiators of the present invention may be in the form of a pharmaceutical composition containing the extract of Lentinus edodes mycelium and optionally a pharmaceutically acceptable carrier.

γδT cell activity enhancers or immunopotentiators of the present invention may be administered in the form of injection or oral, mucosal, gastrointestinal or percutaneous formulation.

γδT cell activity enhancers or immunopotentiators of the present invention may also be in the form of a food, drink or feed.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows that the proportion of γδT cells in peripheral blood increases following administration of the extract of Lentinus edodes mycelium of the present

invention.

FIG. 2 shows the results of flow cytometric analysis of γδT cells before and after administration of the extract of Lentinus edodes mycelium.

FIG. 3 shows that the proportion of αβT cells in peripheral blood decreases following administration of the extract of Lentinus edodes mycelium of the present invention.

THE MOST PREFERRED EMBODIMENTS OF THE INVENTION

An extract of Lentinus edodes mycelium used for enhancing the activity of γδT cells according to the present invention refers to an extract obtained by crushing and decomposing mycelia grown from Lentinus edodes cultured on a solid medium, or a solid medium itself containing Lentinus edodes mycelia in the presence of water and an enzyme.

An extract of Lentinus edodes mycelium used herein is preferably obtained by, but not limited to, the following process. Lentinus edodes spawn is inoculated on a solid medium based on bagasse (sugar cane residue) and defatted rice bran to grow mycelia, and then the solid medium containing the grown mycelia is delignified so that 30% by weight or less is able to pass through a 12-mesh sieve. To this delignified solid medium are added water and one or more enzymes selected from cellulase, protease or glucosidase while maintaining said solid medium at a temperature of 30-55°C, and said solid medium is crushed

and ground in the presence of said enzyme so that at least 70% by weight of bagasse fiber is able to pass through a 12-mesh sieve. Then, the temperature is raised to 95°C to ensure inactivation of the enzyme and sterilization, and the resulting suspension is filtered to give an extract of Lentinus edodes mycelium. The extract of Lentinus edodes mycelium as prepared above may be directly used in γδT cell activity enhancers of the present invention, but conveniently concentrated and freeze-dried into powder to be stored and used in various forms. The freeze-dried product is a brown powder with hygroscopic characteristics and has a peculiar taste and odor.

The extract of Lentinus edodes mycelium was tested for its in vivo γδT cell activity-enhancing effect by the method described in the examples below, and it will be seen from these examples that it has a remarkable in vivo γδT cell activity-enhancing effect.

γδT cell activity enhancers of the present invention are effective for treating and/or preventing tumor induced by tumor cells to which γδT cells are cytotoxic. γδT cell activity enhancers of the present invention are characterized in that they enhance the activity of γδT cells and thereby lead to the destruction of tumor cells under the action of the activated γδT cells, rather than having a direct action on specific tumor cells. Consequently, tumor cells to be treated with γδT cell activity enhancers of the present invention may be not only malignant tumor cells but also benign tumor cells, and are

not limited to specific tumor cells. In addition, γδT cell activity enhancers containing the extract of Lentinus edodes mycelium and optionally a pharmaceutically acceptable carrier can be used in the form of both therapeutic and prophylactic compositions.

γδT cell activity enhancers of the present invention can also be used as therapeutic and/or prophylactic compositions for bacterial or viral infections. γδT cell activity enhancers of the present invention are intended to enhance the activity of γδT cells and thereby to remove infecting bacteria or viruses from the patient rather than to directly act on specific bacteria or viruses. Bacterial or viral diseases that can be treated with γδT cell activity enhancers of the present invention include such diseases as , but are not limited to, those caused by Mycobacterium spp. Listeria monocytogenes, hepatitis viruses (types A, B and C), human immunodeficiency virus, vaccinia virus and the like.

γδT cell activity enhancers of the present invention in the form of a therapeutic and/or prophylactic composition are administered most preferably via the oral route, but may also be administered via intravenous, intraperitoneal, subcutaneous, intramuscular, nasal, percutaneous or other route. Dosage forms suitable for oral administration include, but are not limited to, tablets, capsules, powders, granules, solutions, syrups, etc. Dosage forms suitable for nasal or percutaneous administration include, but not limited to, cataplasms,

patches, etc.

Pharmaceutically acceptable carriers include, but are not limited to, suitable excipients, binders, disintegrators, lubricants, flavoring agents, colorants, solubilizers, suspending agents, coating agents or the like known in the art.

Pharmaceutically acceptable carriers that can be optionally mixed in γδT cell activity-enhancing formulations of the present invention include, but are not limited to excipients such as lactose, dextrose, starch, crystalline cellulose; binders such as starch, gelatin, methyl cellulose, polyvinylpyrrolidone; disintegrators such as starch, calcium carboxymethylcellulose, carboxymethyl starch; lubricants such as talc, stearates; coating agents such as sucrose, talc, gelatin; as well as various brighteners, flavoring agents, colorants, corrigents, solubilizers, stabilizers, suspending agents, absorbefacients or the like known in the art depending on the purpose. For use as injections, various diluents commonly used in this field of art such as water or ethyl alcohol can be used.

The dose of γδT cell activity enhancers of the present invention is determined by physicians taking into account the age, weight and condition of the subject, the route of administration and other factors. The dose is not strictly limited because the extract of Lentinus edodes mycelium contained in γδT cell activity enhancers of the present invention is highly safe, and has been

traditionally ingested as a component of food. For example, the extract of Lentinus edodes mycelium is normally administered preferably at a dose of 100 mg - 10000 mg several times (about 2-3 times) daily (a total of 200 mg - 30000 mg daily), more preferably 500 mg - 5000 mg three times daily (a total of 1500 mg - 15000 mg daily), most preferably 1000 mg - 1500 mg three times daily (a total of 3000 mg - 4500 mg daily). It may be administered in combination with other antitumor agents.

γδT cell activity enhancers of the present invention can be provided in a dosage form also suitable for adoptive immunotherapy for treating tumor. Adoptive immunotherapy refers to a type of anti-tumor therapy intended to kill tumor cells by transferring into a subject sensitized cells, normally lymphocytes. In the case of the present invention, γδT cells are initially isolated from peripheral blood of the subject, and the isolated γδT cells are activated in vitro by a γδT cell activity enhancer of the present invention, and then the activated γδT cells are returned into the subject. As a result, tumor cells in the subject can be destroyed by the action of the activated γδT cells.

γδT cell activity enhancers of the present invention may be the extract of Lentinus edodes mycelium itself or pharmaceutical or veterinary compositions comprising a γδT cell activity enhancer containing the extract of Lentinus edodes mycelium and a pharmaceutically acceptable carrier.

γδT cell activity enhancers of the present invention can also be provided in the form of a food. Preferred

forms of food include powders, granules, pastes, jellies, etc. Granules desirably are supplemented with sugars such as lactose to add a sweet taste. γδT cell activity enhancers of the present invention can also be provided in the form of a drink. These foods or drinks may be supplemented with vitamins, minerals such as calcium, alcohols, deodorants such as polyphenols in addition to the extract of Lentinus edodes mycelium. These foods or drinks include the categories of specific health foods, medical foods or the like.

γδT cell activity enhancers of the present invention can also be provided in the form of a feed or feed additive. γδT cell activity enhancers of the present invention can be used as a feed or feed additive for domestic animals to treat and/or prevent tumor occurring in domestic animals or to treat and/or prevent bacterial or viral infections in domestic animals. As a result, the amount of therapeutic agents such as antibiotics currently used can be reduced, thereby reducing farming costs. Another advantage is that the period during which shipment of animals is suspended due to the administration of antibiotics can be shortened.

In vivo γδT cell activity-enhancing effect was tested in human subjects as follows. Human subjects initially received 3.6 g of the extract of Lentinus edodes mycelium bulk powder daily for 7 days (a total of 25.2 g). Then, the proportion of γδT cells in peripheral blood after administration of the extract of Lentinus edodes mycelium was determined by flow cytometry as compared with the

proportion before administration.

The following examples, which further illustrate the present invention, should not be taken as limiting the the scope of the invention thereto. Various changes and modifications can be made by those skilled in the art and such changes and modifications are also included in the scope of the present invention.

EXAMPLES

Example 1: Preparation of an extract of Lentinus edodes mycelium

A solid medium consisting of 90 parts by weight of bagasse and 10 parts by weight of rice bran was soaked with an appropriate amount of pure water, and then inoculated with Lentinus edodes spawn and allowed to stand in an incubator at controlled temperature and humidity to grow mycelia. After mycelia spread over the solid medium, the bagasse base was delignified so that 24% by weight or less may pass through a 12-mesh sieve. To 1.0 kg of this delignified medium were added 3.5 L of pure water and 2.0 g of purified cellulase while maintaining the solid medium at 40°C to prepare a medium-containing mixture.

Then, the medium-containing mixture was circulated by a variable speed gear pump, during which the solid medium was crushed and ground under the gears for about 200 minutes so that about 80% by weight of bagasse fiber may pass through a 12-mesh sieve. The medium-containing mixture was crushed and ground while the temperature of

said mixture was gradually increased. Then, the medium-containing mixture was further heated to 90°C and allowed to stand for 30 minutes. This heating to 90°C ensured inactivation of the enzyme and sterilization. The resulting medium-containing mixture was filtered through a 60-mesh filter cloth to give an extract of Lentinus edodes mycelium solution, which was concentrated and then freeze-dried into an extract of Lentinus edodes mycelium bulk powder.

The extract of Lentinus edodes mycelium as prepared above contained 25.3% (w/w) carbohydrates determined by the phenol-sulfuric acid method, 19.7% (w/w) proteins determined by the Lowry method and 2.6% (w/w) polyphenols determined by the Folin-Denis method using gallic acid as standard. The extract of Lentinus edodes mycelium further contains 8% crude fat, 22% crude ash and about 20% soluble nitrogen-free materials other than carbohydrates.

The extract of Lentinus edodes mycelium had a sugar composition (%) as follows: Xyl 15.2, Ara 8.2, Man 8.4, Gul 39.4, Gal 5.4, GlcN 12.0, GluUA 11.3.

Example 2: In vivo γδT cell activity-enhancing test of the extract of Lentinus edodes mycelium

Three human subjects (subjects A-C) received orally 3.6 g/day of the extract of Lentinus edodes mycelium bulk powder daily for 7 days (a total of 25.2 g). After the period of administration of the extract of Lentinus edodes mycelium, peripheral blood was collected from the human

subjects. The proportion of γδT cells in peripheral blood collected after administration was determined by flow cytometry as compared with the proportion in peripheral blood collected before administration. The results are shown in Figs. 1 and 2.

In all of the three subjects, the proportion of γδT cells in peripheral blood increased by an average of 40% or more after administration of the extract of Lentinus edodes mycelium as compared with before administration.

Table 1: Increase of the proportion of γδT cells in peripheral blood after administration

	Subject A	Subject B	Subject C	Mean ± SEM
Increase	124.39%	146.15%	150.00%	140.18%±7.97%

Peripheral blood collected from the same subject before and after administration was tested for other markers than γδT cells. As a result, the extract of Lentinus edodes mycelium showed no proliferative activity on αβT cells and on average the proportion of αβT cells decreased (Fig. 3).

INDUSTRIAL APPLICABILITY

γδT cell activity enhancers containing the extract of Lentinus edodes mycelium of the present invention were found to actually activate γδT cells. Thus, γδT cell activity enhancers of the present invention can be used for preventing or treating tumor, bacterial infections and

viral infections because they have the effect of protecting living bodies against tumor, bacterial infections and viral infections by inducing cytotoxic activity against tumor cells, antibacterial activity and antiviral activity of γδT cells. Moreover, γδT cell activity enhancers of the present invention are suitable for wide industrial application because they can be used safely without side effects.

They can also be used in domestic animals with bacterial and/or viral infections to reduce the amount of therapeutic agents currently used such as antibiotics, thereby reducing costs for raising. They also have the advantage that the period during which shipment is suspended can be shortened because antibiotics are not used.

CLAIMS

1. A γδT cell activity enhancer containing an extract of Lentinus edodes mycelium.

2. A pharmaceutical or veterinary γδT cell activity enhancer comprising an extract of Lentinus edodes mycelium and a pharmaceutically acceptable carrier.

3. The γδT cell activity enhancer of claim 1 or 2 for oral administration.

4. The γδT cell activity enhancer of claim 1 in the form of a food.

5. The γδT cell activity enhancer of claim 1 in the form of a drink.

6. The γδT cell activity enhancer of claim 1 in the form of a feed.

7. The γδT cell activity enhancer of claim 1 or 2 for injection or percutaneous absorption.

8. The γδT cell activity enhancer of claim 1 for in vitro activating γδT cells collected from a subject.

9. The γδT cell activity enhancer of any one of claims 1 to 8 used for treating tumor.

10. The γδT cell activity enhancer of any one of claims 1 to 8 used for preventing or treating a bacterial infection or a viral infection.

11. A method for treating a tumor of a subject by administering in vivo the γδT cell activity enhancer of claim 9.

12. A method for preventing or treating a bacterial infection or a viral infection in a subject by

administering in vivo the γδT cell activity enhancer of claim 10.

13. A use of an extract of Lentinus edodes mycelium for the preparation of a γδT cell activity enhancer.

ABSTRACT

The present invention develops and provides γδT cell activity enhancers and therefore immunopotentiators containing an extract of Lentinus edodes mycelium for use in the treatment of tumor or the treatment and/or prevention of bacterial infections or viral infections by taking advantage of the effect that the extract of Lentinus edodes mycelium has in remarkably enhancing the activity of γδT cells.

FIG. 1

09/856717

FIG. 2

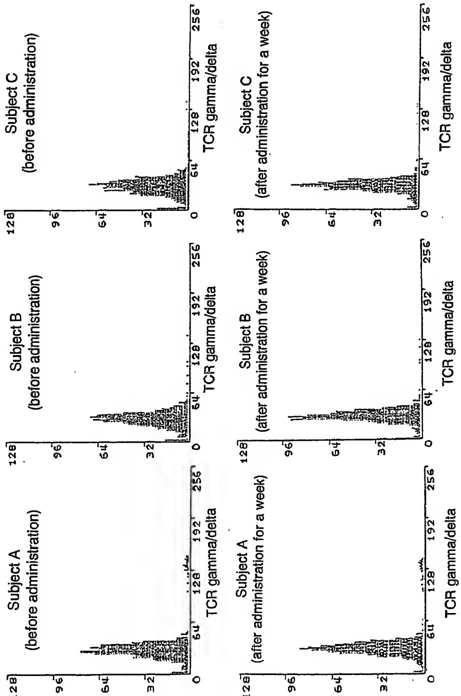

FIG. 3

BIRCH, STEWART, KOLASCH & BIRCH, LLP

PLEASE NOTE: YOU MUST COMPLETE THE FOLLOWING:

COMBINED DECLARATION AND POWER OF ATTORNEY

FOR PATENT AND DESIGN APPLICATIONS

ATTORNEY DOCKET NO. 0230-0162P

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated next to my name; that I verily believe that I am the original, first and sole inventor (if only one inventor is named below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Insert Title: ➡ γδ T CELL IMMUNOACTIVITY ENHANCERS CONTAINING EXTRACT OF LENTINUS EDODES MYCELIUM

Fill in Appropriate Information - For Use Without Specification Attached: ➡

the specification of which is attached hereto. If not attached hereto,

the specification was filed on _____ as
United States Application Number _____; and /or

the specification was filed on November 26, 1999 as PCT
International Application Number PCT/JP99/06617; and was
amended under PCT Article 19 on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I do not know and do not believe the same was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months (six months for designs) prior to this application, and that no application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representatives or assigns, except as follows.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Insert Priority Information: (if appropriate) ➡

Prior Foreign Application(s)

(Number)	(Country)	(Month/Day/Year Filed)	Priority Claimed	
337822/1998	Japan	11/27/98	☒ Yes	☐ No
			☐ Yes	☐ No
			☐ Yes	☐ No
			☐ Yes	☐ No
			☐ Yes	☐ No

Insert Provisional Application(s): (if any) ➡

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

(Application Number) (Filing Date)

(Application Number) (Filing Date)

Insert Requested Information: (if appropriate) ➡

All Foreign Applications, if any, for any Patent or Inventor's Certificate Filed More Than 12 Months (6 Months for Designs) Prior To The Filing Date of This Application:

Country	Application No.	Date of Filing (Month/Day/Year)

Insert Prior U.S. Application(s): (if any) ➡

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Number) (Filing Date) (Status - patented, pending, abandoned)

(Application Number) (Filing Date) (Status - patented, pending, abandoned)

I hereby appoint the following attorneys to prosecute this application and/or an international application based on this application and to transact all business in the Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the attorneys identified below, unless the inventor(s) or assignee provides said attorneys with a written notice to the contrary:

16

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PLEASE NOTE: YOU MUST COMPLETE THE FOLLOWING:

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00

Full Name of First or Sole Inventor: Insert Name of Inventor; Insert Date This Document is Signed; Insert Residence; Insert Citizenship; Insert Post Office Address

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* DATE OF SIGNATURE